



## RNA-seq Gene Profiling Reveals Transcriptional Changes in the Late Phase during Compatible Interaction between a Korean Soybean Cultivar (*Glycine max* cv. Kwangan) and *Pseudomonas syringae* pv. *syringae* B728a

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Soybean (*Glycine max* (L) Merr.) provides plant-derived proteins, soy vegetable oils, and various beneficial metabolites to humans and livestock. The importance of soybean is highly underlined, especially when carbon-negative sustainable agriculture is noticeable. However, many diseases by pests and pathogens threaten sustainable soybean production. Therefore, understanding molecular interaction between diverse cultivated varieties and pathogens is essential to developing disease-resistant soybean plants. Here, we established a pathosystem of the Korean domestic cultivar Kwangan against *Pseudomonas syringae* pv. *syringae* B728a. This bacterial strain caused apparent disease symptoms and grew well in trifoliolate leaves of soybean plants. To examine

the disease susceptibility of the cultivar, we analyzed transcriptional changes in soybean leaves on day 5 after *P. syringae* pv. *syringae* B728a infection. About 8,900 and 7,780 differentially expressed genes (DEGs) were identified in this study, and significant proportions of DEGs were engaged in various primary and secondary metabolisms. On the other hand, soybean orthologs to well-known plant immune-related genes, especially in plant hormone signal transduction, mitogen-activated protein kinase signaling, and plant-pathogen interaction, were mainly reduced in transcript levels at 5 days post inoculation. These findings present the feature of the compatible interaction between cultivar Kwangan and *P. syringae* pv. *syringae* B728a, as a hemibiotroph, at the late infection phase. Collectively, we propose that *P. syringae* pv. *syringae* B728a successfully inhibits plant immune response in susceptible plants and de-regulates host metabolic processes for their colonization and proliferation, whereas host plants employ diverse metabolites to protect themselves against infection with the hemibiotrophic pathogen at the late infection phase.

**Keywords** : bacterial brown spot, compatible interaction, mRNA-seq, *Pseudomonas syringae* pv. *syringae* B728a, soybean

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Soybean (*Glycine max* (L) Merr.), an edible legume, is one of the most important crops as an excellent source of plant-based oils, vegetable proteins, and secondary metabolites. Wild soybean (*G. soja*) was domesticated to *G. max* in Northeast Asia, including Korea, China, and Japan. So far, over 60,000 varieties have been developed and culti-

vated in different countries (Carter et al., 2004; Li et al., 2020). Since a reference genome of cultivated accession Williams 82 was published, functional studies of soybean are accelerating, and the pan-genome of soybean was also analyzed (Liu et al., 2015b, 2020; Schmutz et al., 2010; Wang and Tian, 2015). A Korean soybean cultivar (cv.), Kwangan, also known as 'Kwangankong' (Suwon No. 159), was bred by crossing a high-protein Japanese cv. Dongsan (Touzan) 69 with a virus disease-resistant Korean cv. Hwangkeum (Suwon No. 97), and largely cultivated in the Central and Southern regions of Korea (Kim et al., 2011; Lee et al., 2015; Soybean Breeding Team, Upland Crop Div., Crop Experiment and Experiment Station, 1994). The soybean cv. Kwangan exhibits good crop traits such as high protein content and seed yield and relatively higher resistance against pests and pathogens than other Korean sprout soybean cultivars (Kim et al., 2011; Soybean Breeding Team, Upland Crop Div., Crop Experiment and Experiment Station, 1994).

Like other crops and vegetables, the productivity and quality of soybean rely on disease management. Indeed, the average soybean yield loss due to pests and pathogens was 21.4% (11.0-32.4%) from 2010 to 2014 globally (Savary et al., 2019). Worldwide, cyst nematode (*Heterodera glycines*), white mold (*Sclerotinia sclerotiorum*), and soybean rust (*Phakopsora pachyrhizi*) are the most destructive pathogens in soybean, and many fungi, oomycetes, and nematodes also cause severe yield loss of soybean (Savary et al., 2019). Additionally, bacterial blight and bacterial pustule, the major bacterial diseases in fields, are caused by *Pseudomonas syringae* pv. *glycinea* and *Xanthomonas axonopodis* pv. *glycines*, respectively (Faske et al., 2014; Hartman et al., 2015). Even though agricultural practices such as aggressive usage of agricultural chemicals, crop rotation, and tillage, have been performed to avoid primary and secondary inoculum from previously infected crop residues, disease management of soybean is primarily dependent on cultivating disease-resistant plants (Faske et al., 2014). Thus, considering eco-friendly and sustainable food supply, previously unidentified genetic resources have to be unveiled and applied to molecular breeding to increase disease resistance in soybean plants.

*P. syringae* pv. *syringae* among more than 60 pathovars of *P. syringae* exhibits polyphagous bacterium with a broad-spectrum host range (Kennelly et al., 2007). *P. syringae* pv. *syringae* can infect soybean leaves and pods, and consequently cause necrotic lesions surrounded by chlorotic zones (Gnanamanickam and Ward, 1982). The genome sequence of *P. syringae* pv. *syringae* B728a (*PssB728a*), a representative strain of *P. syringae* pv. *syringae* that

was firstly isolated from a snap bean (*Phaseolus vulgaris*) leaflet in Wisconsin, USA, was complete (Feil et al., 2005; Loper and Lindow, 1987). *PssB728a* retains an effector repertoire secreted into its host plants through its type III secretion system (Vinatzer et al., 2006). In the field, this bacterium can multiply and survive for many generations on the (non)host leaf surfaces as an epiphyte (Ercolani et al., 1974; Hirano and Upper, 1990; Lindemann et al., 1984). The size of the epiphytic population increases upon heavy rains, promoting the bacteria's rapid multiplication (Hirano et al., 1994, 1996). Thus, it is considered an intriguing source to study the molecular interaction between host plants and parasitic microbes (Vinatzer et al., 2006).

Recently, (molecular) components of soybeans associated with biotic stress response have been identified (Cooper et al., 2013; Delgado-Cerrone et al., 2018; Helm et al., 2019; Liu et al., 2015b; Pottinger et al., 2020; Russell et al., 2015; Wei et al., 2020; Yuan et al., 2020). Thus, we felt it is necessary to fill the knowledge gap in a Korean soybean accession. In this study, we evaluated the bacterial disease in the soybean cv. Kwangan inoculated with *PssB728a* and analyzed transcriptional change in soybean plants to identify genes involved in pathogenesis and immune response against a virulent *Pseudomonas* infection. In the infected soybean leaves, 8,902 and 7,786 genes were up- and down-regulated, respectively, at the late phase of infection. They appeared to be associated and involved in mainly biological pathways, including metabolic pathways, secondary metabolites, hormonal signal transduction, and plant-pathogen interaction upon *PssB728a* bacterial infection. Taken together, this pathosystem shows that the expression of soybean orthologs to well-known plant immune-related genes, especially in plant hormone signal transduction, *MITOGEN-ACTIVATED PROTEIN KINASE* (MAPK) signaling, and plant-pathogen interaction, is reduced during a compatible interaction. In contrast, soybean genes involved in the biosynthesis of primary and secondary metabolites are strongly expressed by *PssB728a* infection. This finding can help understand how *PssB728a* suppresses the plant immunity in the susceptible cultivar. We propose a few signaling pathways primarily affected by a virulent bacterial infection during a compatible interaction.

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## Materials and Methods

**Plant material and growth conditions.** *G. max* cv. Kwangan was used for this study. Soybean seeds were sterilized with chlorine gas (100 ml of 5.25% NaOCl supplemented with 3 ml of 12 N HCl) in a fume-hood overnight and sowed on soils (Nongwoo Bio, Suwon, Korea). Soybean

plants grew under long-day conditions (25°C, 16 h light/8 h dark, 120  $\mu\text{mol}/\text{m}^2/\text{s}$ ) in a walk-in growth chamber.

**Bacterial strain and inoculation.** The second trifoliolate leaves of 3-week-old soybean plants were inoculated with *PssB728a*. We sprayed *PssB728a* suspension ( $\text{OD}_{600} = 0.01$ ) in 10 mM  $\text{MgSO}_4$  containing 0.01% Silwet L-77 to the abaxial and adaxial side of leaves and kept the infected plants in the dark conditions with high humidity during the indicated periods. At least eight leaf discs were taken from infected leaves, and the number of bacteria in the leaf disc was counted using the typical serial dilution method. To analyze the epiphytic growth of *PssB728a* in plants, leaf discs of infected leaves with *PssB728a* were washed thoroughly with 10 mM  $\text{MgSO}_4$ , in which bacterial growth was counted. The washed leaves were then soaked in 70% ethanol during several seconds for surface sterilization and were ground in 10 mM  $\text{MgSO}_4$  to measure the endophytic growth of *PssB728a* (Lee et al., 2012).

**Transcriptome analysis.** Trifoliolate leaf tissues of soybean plants (~1 g) were harvested on the 5 days after spray-inoculation with 10 mM  $\text{MgSO}_4$  (2 of the control group) and *PssB728a* ( $\text{OD}_{600} = 0.01$ ) (2 of treatment group) for the extraction of total RNA. RNA purification using RNeasy spin column (Qiagen, Hilden, Germany) was then conducted following the manufacturer's instruction. After confirming the RNA quality (Bioanalyzer, Agilent, Palo Alto, CA, USA), the total RNA was subject to construct mRNA sequencing libraries using a TruSeq Stranded Total RNA LT Sample Prep Kit (Plant) according to the manufacturer's instruction (Illumina, San Diego, CA, USA) (Martin and Wang, 2011). Each library from two biological replicates per treatment (total 4 libraries) was sequenced for 101 bp paired-end reads with a HiSeq 2500 platform (Illumina) serviced by Macrogen in Seoul, Korea.

The RNA-seq data were processed by TopHat2 and Bowtie2 (Langmead and Salzberg, 2012; Trapnell et al., 2009). After quality control of raw sequence reads, provided by Phred quality score and FastQC, adaptor sequences were trimmed using the Trimmomatic program to generate trimmed data (Bolger et al., 2014). The trimmed reads were aligned and assembled with the *G. max* v2.1 gene model ([https://www.ncbi.nlm.nih.gov/assembly/GCA\\_000004515.4/](https://www.ncbi.nlm.nih.gov/assembly/GCA_000004515.4/)) using the HISAT2 and StringTie (Kim et al., 2019; Pertea et al., 2015, 2016). The raw read counts, FPKM (fragments per kilobase of transcript per million mapping reads), TPM (transcripts per million), and simple description of the mapped transcripts to known soybean genes were presented in Supplementary Table 1.

Followed by the normalization by trimmed mean of M-values in edgeR, differentially expressed genes (DEGs) in infected plants were determined by two statistical outputs ( $|\text{fold change (FC)}| \geq 2$  and exactTest raw.  $P < 0.05$ ) compared with the mock-inoculated plants (Chen et al., 2016). FC (infected samples/non-infected samples) and related statistical data of each DEG were listed in Supplementary Table 2.

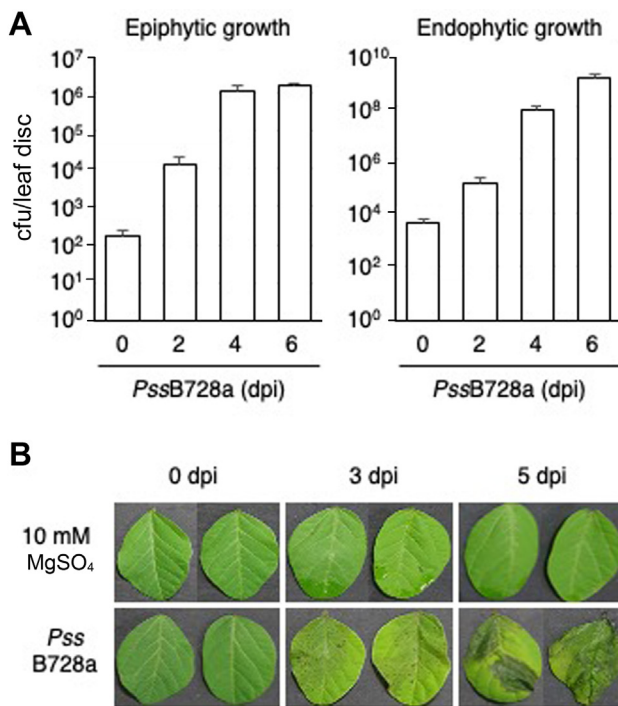
**Functional classification and enrichment analyses of DEGs.** The DEGs were used for gene ontology (GO) enrichment analysis using Protein Analysis Through Evolutionary Relationships (PANTHER) (Mi et al., 2019; Thomas et al., 2003) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (Kanehisa et al., 2007). The GO terms and KEGG pathways with  $P < 0.01$  and  $Q < 0.01$  were considered to be significantly enriched in DEGs. The list of enriched GO terms and KEGG pathways related to each DEG were shown in Supplementary Tables 3 and 4. We can share detailed analysis procedures under a request.

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## Results and Discussion

The soybean cv. Kwangan owns good agronomic features such as high resistance to soybean mosaic virus and high protein quality (Kim et al., 2011; Lee et al., 2015; Soybean Breeding Team, Upland Crop Div., Crop Experiment and Experiment Station, 1994). Therefore, the cv. Kwangan was broadly cultivated in Korea and used as a well-established parental plant to develop the Biotech soybean (Kim et al., 2012; Yeom et al., 2020). However, the molecular response of cv. Kwangan to bacterial disease, especially bacteria brown spot caused by *P. syringae* pv. *syringae*, has not been done. This study focused on the transcriptional changes in susceptible response in soybean plants upon *PssB728a* infection.

**Growth of *P. syringae* pv. *syringae* B728a in trifoliolate leaves of soybean plants.** *PssB728a* exploits two different habitats (leaf surface and apoplast) as a phytopathogenic bacterium. The epiphytic *PssB728a* population acts as an inoculum to infect host plants successfully (Hirano and Upper, 2000). The epiphytic bacterial growth was counted every 2 days after a spray-inoculation of *PssB728a*. The number of bacteria on the leaf surface increased 2 days post inoculation (dpi), and the bacterial growth was saturated on the 4th day and 6th day (4 and 6 dpi) with populations of  $10^6$  colony forming unit (cfu) per leaf disc (Fig. 1A, left). Consistently, the endophytic bacterial population reached >



**Fig. 1.** Disease symptom development and bacterial growth in leaves of soybean cv. Kwangan infected with *Pseudomonas syringae* pv. *syringae* (*Pss*) B728a. (A) The epiphytic and endophytic bacterial growths were tested on infected soybean leaves. At least eight leaf discs were used to count the endophytic and epiphytic bacterial growth of *Pss*B728a on the indicated day post inoculation (dpi). The bars present the mean  $\pm$  standard error. (B) The second trifoliolate leaves from 3-week-old soybean plants grown in long-day conditions were spray-inoculated with 10 mM MgSO<sub>4</sub> (mock) and *Pss*B728a (OD<sub>600</sub> = 0.01) and incubated under dark conditions with high humidity and 25°C during the experiment. Disease symptoms were taken in photographs 0, 3, and 5 dpi.

10<sup>8</sup> cfu per leaf disc at 4 dpi (Fig. 1A, right), and the lesion increased in size as time progressed (Fig. 1B). The bacterial growth assay reveals that *Pss*B728a causes a compatible interaction with soybean cv. Kwangan.

#### DEGs during *P. syringae* pv. *syringae* B728a infection.

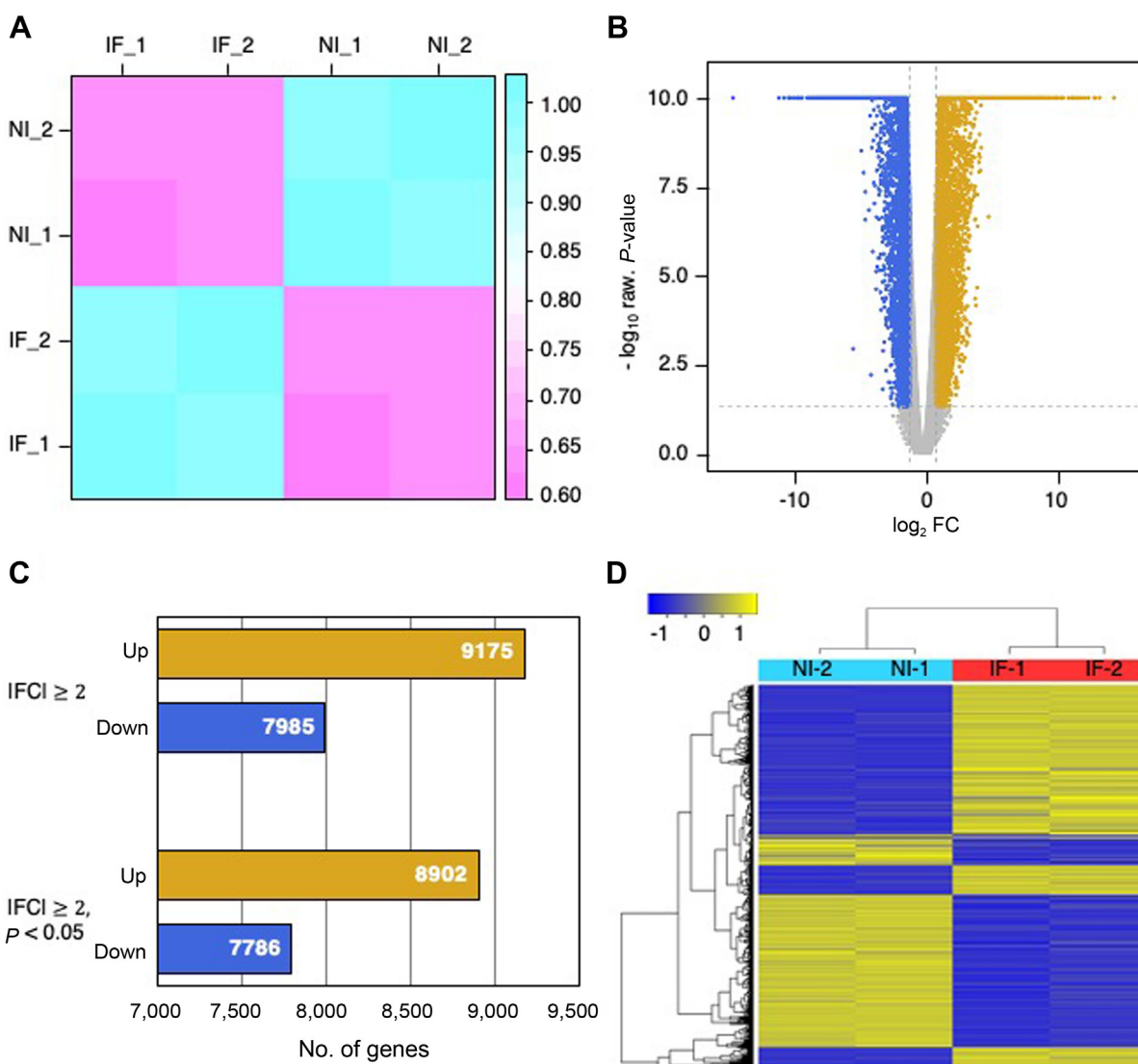
Transcriptome sequencing libraries were made with total RNA from soybean leaves collected on day 5 after inoculation with 10 mM MgSO<sub>4</sub> (non-infected sample, NI) and *Pss*B728a (infected sample, IF). Among 60,506 known soybean transcripts, 31,608 genes, except 28,898 not detected in at least one sample, were used in the transcriptome analysis. The correlation matrix of Pearson's coefficient among four samples (NI\_1, NI\_2, IF\_1, and IF\_2) indicated that two independent biological replicates were highly reproducible (Fig. 2A). In addition, multidimensional scal-

ing analysis showed that NIs and IFs were separately clustered, and components I and II reflected a 97.4% and 2.1% difference among samples, respectively (data not shown). These statistical analyses show that the transcriptome-seq data can represent the transcriptional reprogramming occurring in infected soybean leaves with *Pss*B728a at 5 dpi.

In a volcano plot, the up-regulated and down-regulated genes with  $|FC| \geq 2$  and  $P < 0.05$  were displayed on the right (brown dots) and the left (blue dots) sides, respectively (Fig. 2B). About 8,900 up-regulated genes (14.7% of known soybean transcripts) and 7,780 down-regulated genes (12.9%) were assigned as DEGs in infected leaves with *Pss*B728a (Fig. 2C). Hierarchical clustering of expression levels of DEGs (up to 3,000 of 16,680 genes) indicated that two biological replicates for non-infected plants and infected plants had also been done reproducibly (Fig. 2D). Therefore, we suggest that DEGs' information obtained from the transcriptome analysis helps us to understand the transcriptional changes at 5 dpi in susceptible soybean plants to *Pss*B728a infection. Whole lists of DEGs were presented in Supplementary Table 2.

**The enriched GO terms of DEGs.** The cellular and physiological processes of plants are altered upon pathogen infection. Molecular networks composed of complex interactions between/among genes, proteins, and metabolic compounds are involved especially in plant immune signaling, transcription of immune-related genes, and biosynthesis of phytohormones and secondary metabolites, as the outcome of a plant-microbe interaction (Delplace et al., 2022). A compatible interaction between host and pathogen leads to successful infection and disease (Glazebrook, 2005). Different from an incompatible interaction accompanying a hypersensitive response due to intensified immune responses, the compatible interaction entails interruption of plant immunity by effectors and toxins derived from pathogens, resulting in suppression of immune responses during pathogenesis (Chisholm et al., 2006; Dangl and Jones, 2001; Dodds and Rathjen, 2010; Jones and Dangl, 2006). To analyze which biological processes and molecular functions of DEGs were implicated in the compatible interaction between cv. Kwangan and *Pss*B728a, the GO enrichment analysis was accomplished, which explains the associations between gene products and GO terms using the PANTHER classification system.

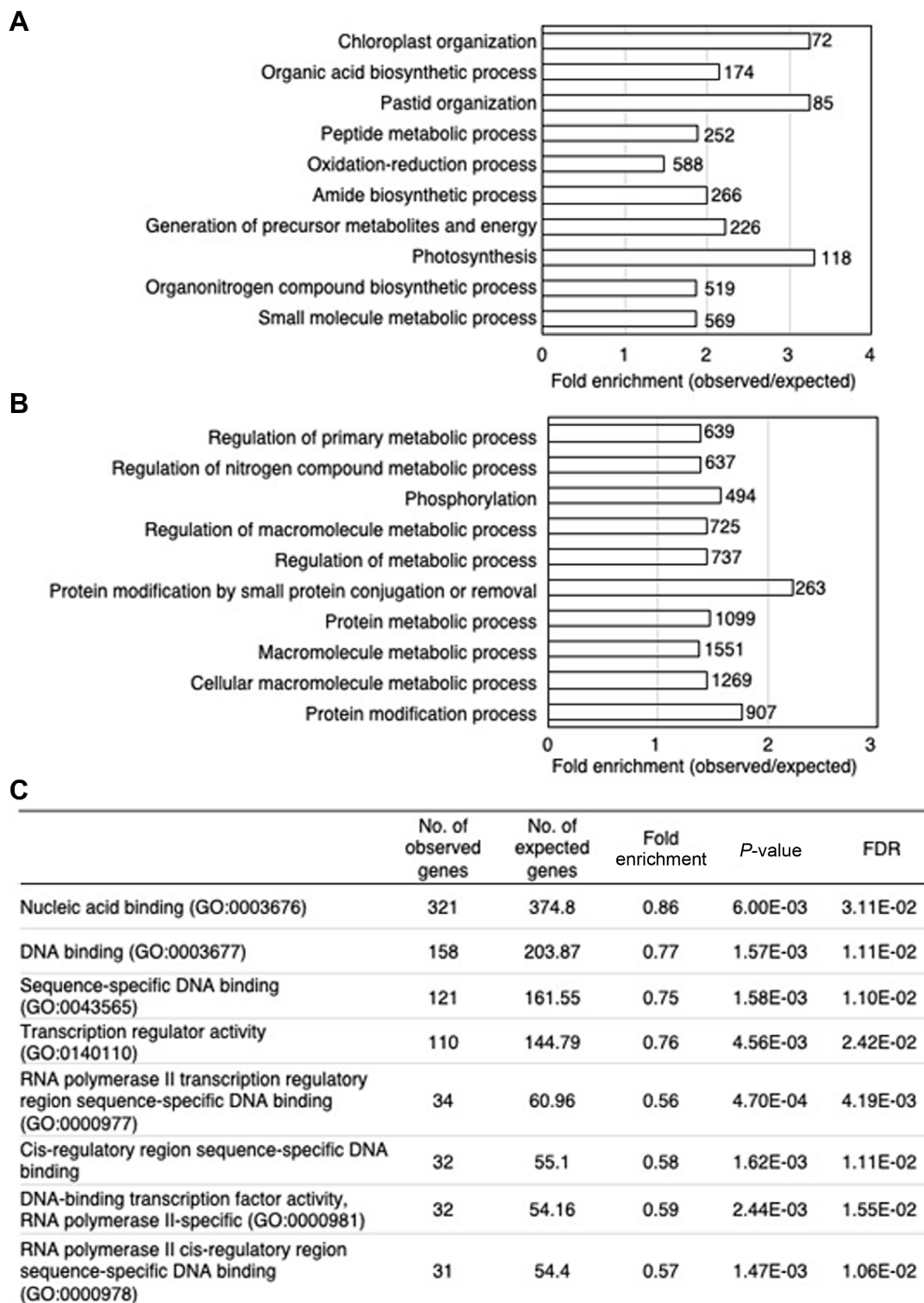
First, all up-regulated and down-regulated genes were subjected to GO analysis (GO biological process) to point out biological processes in which DEGs are involved. The top 10 categories exhibiting the lowest  $P$ -values were listed with the fold enrichments determined by the ratio of the



**Fig. 2.** Quality of RNA-seq analysis and number of differentially expressed genes (DEGs) in infected soybean leaves with *Pseudomonas syringae* pv. *syringae* B728a. (A) Correlation matrix of Pearson's coefficient ( $-1 \leq r \leq 1$ ) to determine the similarity of each sample. NI and IF mean samples from non-infected and *PssB728a*-infected leaves of soybean plants, respectively. A closer value to  $r = 1$  presents a higher similarity between samples. (B) A volcano plot to verify DEGs in *PssB728a*-infected plants versus non-infected soybean. Blue and brown dots indicate genes displaying fold change (FC)  $\leq -2$  and  $\geq 2$  with  $P < 0.05$ , respectively. Vertical dotted lines showed the  $|FC| = 2$  and a horizontal dotted line indicates  $P = 0.05$ . (C) Numbers of genes whose FC is up- and down-regulated on day 5 after *PssB728a* infection. (D) Heat map showing the expression pattern of DEGs in two biological replications of non-infected (NI) leaves and *PssB728a*-infected (IF) leaves. Genes are grouped according to hierarchical clustering analysis (Euclidean distance, complete linkage). Rows are clustered using distance and average linkage. The color gradient represents the Z-score for  $\log_2$  FC-based normalized values.

number of observed genes versus that of expected genes (Fig. 3A and B). The up-regulated DEGs were enriched in 'small molecule metabolic process (GO:0044281)', 'child terms of organonitrogen compound metabolic process (GO:1901564) and cellular amide metabolic process (GO:0043603)', and 'generation of precursor metabolites and energy (GO:0006091)' (Fig. 3A). On the other hand,

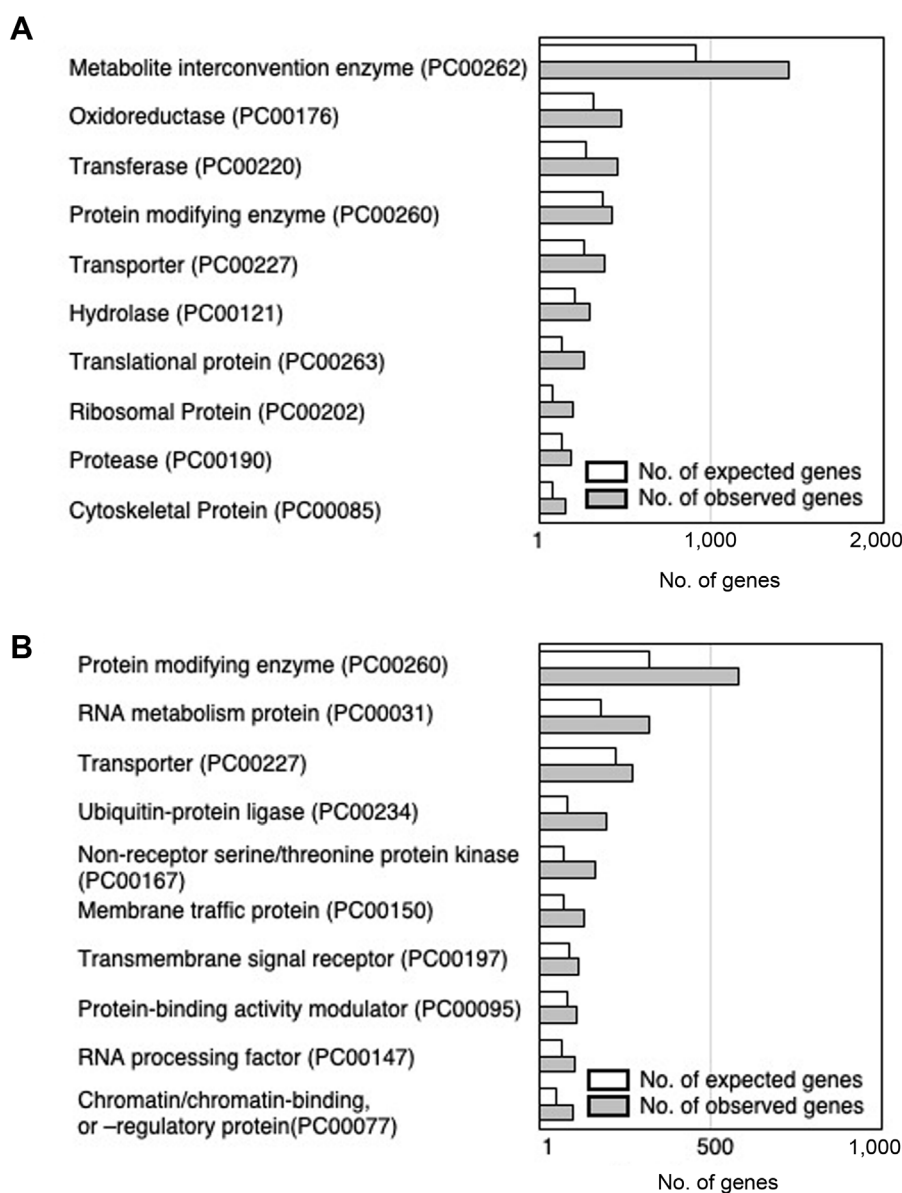
certain kinds of genes involved in 'regulation of metabolic process (GO:0019222)', 'macromolecule metabolic process (GO:0043170)', and 'protein modification process (GO:0006464)' were significantly down-regulated by *PssB728a* at 5 dpi (Fig. 3B). In addition, large sets of genes associated with RNA biological process appeared to be influenced by pathogen infection, because the expression of



**Fig. 3.** Enriched gene ontology (GO) terms of differentially expressed genes (DEGs) in infected soybean plants. (A, B) Top 10 GO biological process terms based on the number of observed genes in this analysis among up-regulated DEGs (A) and down-regulated DEGs (B). The numeric on the right side of the bars shows the exact numbers of observed genes. Fold enrichment was calculated by the ratio of observed gene versus expected gene. (C) Terms of GO-slim molecular function showing fold enrichment  $< 1$ ,  $P < 0.005$ , and  $Q < 0.05$  in up-regulated DEGs. The whole GO biological process, cellular component, and molecular function terms for each DEG were presented in Supplementary Table 3. FDR, false discovery rate.

those genes involved in RNA biosynthesis, RNA metabolic process, mRNA metabolic process, and regulation of RNA biosynthetic process decreased upon bacterial infection (Supplementary Table 3). In the same line with it, the number of observed genes related to transcription was significantly less in the infected plants compared with that of expected genes of the soybean genome (Fig. 3C). Supporting

this, the expression of 99 genes which are implicated in the metabolism of purines (heterocyclic aromatic organic compound consisting of two rings fused, adenine and guanine here), essential building block components for DNA and RNA, was altered by *PssB728a* infection (Supplementary Table 4). Second, we also analyzed the molecular function of DEGs' products (GO molecular function and GO-slim



**Fig. 4.** Enriched gene ontology (GO) protein class terms of differentially expressed genes (DEGs) in infected soybean plants. (A, B) Top 10 GO protein class terms based on the number of observed genes in this analysis among up-regulated DEGs (A) and down-regulated DEGs (B). Up-regulated (8,902 genes, A) and down-regulated (7,786 genes, B) genes were applied to GO enrichment protein class analysis. For each protein, class GO term, the expected (empty) and observed gene (filled) numbers whose *P*-values are lower than 0.01 (statistical significance) are presented. No. of observed genes, number of DEGs associated with a GO term for protein class; no. of expected genes, number of genes expected for each GO term in the genome after normalization of the whole number of genes analyzed in this analysis (8,902 and 7,786).

molecular function). Both up- and down-regulated DEGs were significantly enriched in 62 GO terms, including transferase activity, catalytic activity, kinase activity, and small molecule binding (Supplementary Table 3). These results suggest that primary and secondary metabolisms are mainly affected by *PssB728a* infection, whereas transcriptional activity decreased at 5 dpi.

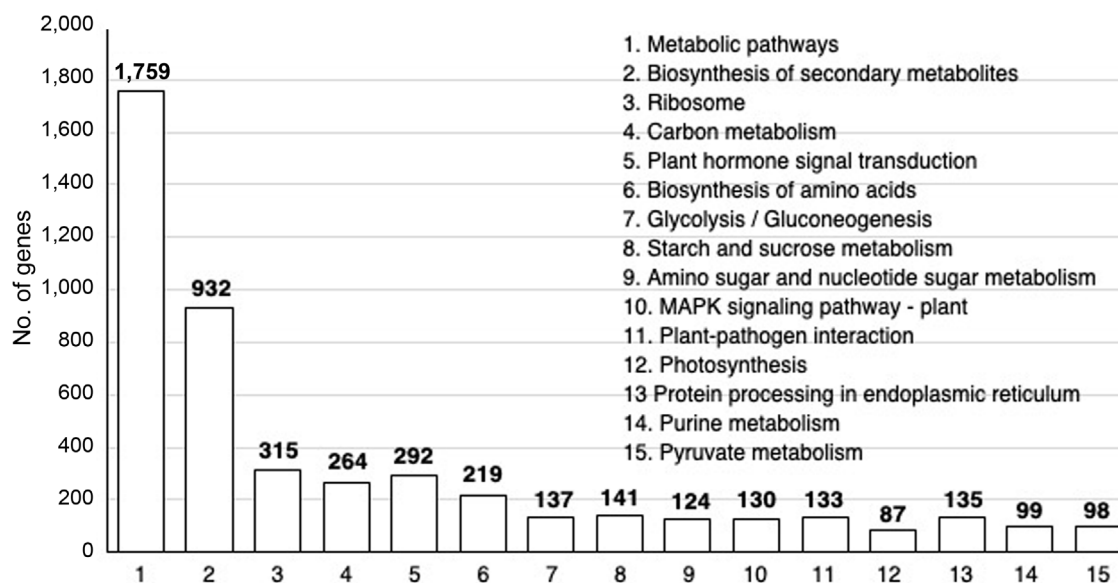
Numerous genes encoding enzymes such as hydrolases, lyases, transferase, and metabolite interconversion enzymes were expressed highly upon bacterial infection (Fig. 4A). In contrast, a large number of genes encoding kinases, especially non-receptor serine/threonine protein kinases (about more than 150 genes) and protein modifying enzymes such as ubiquitin-protein ligases, expressed lower in infected soybean plants than mock-inoculated plants (Fig. 4B). In fact, KEGG pathway analysis revealed that the high population of DEGs was mostly associated with metabolic pathways (carbonate, secondary metabolite, starch and sucrose, amino sugar metabolism) and protein processing (Supplemental Table 4).

**The KEGG pathways related to DEGs.** Transcriptional profiles of several crop plants after pathogen infections clarified particular metabolic and signaling pathways tightly related to compatible and incompatible interaction (Cregeen et al., 2015; Gyetvai et al., 2012; Meng et al.,

2021; Sonah et al., 2016; van Esse et al., 2009; Wang et al., 2010).

Using 3,775 genes mapped in KEGG pathways (<http://www.kegg.jp/kegg/pathway.html>) among 16,688 DEGs, gene-set enrichments were analyzed to examine which pathways were activated or suppressed at 5 dpi in infected leaves (Kanehisa et al., 2022). Fig. 5 presents the top 15 pathways with  $P$ -values  $< 7.47E-45$  and  $Q$ -values  $< 6.63E-44$  as follows; metabolic pathways, biosynthesis of secondary metabolite, carbon metabolism, plant hormone signal transduction, MAPK signaling pathway, plant-pathogen interaction, photosynthesis, protein processing in the endoplasmic reticulum (ER), purine metabolism, pyruvate metabolism, etc. Many genes were mapped simultaneously into different pathways. For example, among 137 genes in the category of glycolysis [7], whose expressions were affected upon bacterial infection, 49 genes (about 36%) participate in the pyruvate metabolism [15]; 50% of genes (49 genes) in pyruvate metabolism work at glycolysis-related pathway as well.

Cellular detoxification of reactive oxygen species (ROS) formed from different metabolic pathways and upon various unfavorable stress conditions is an essential biological process in living organisms to maintain homeostasis (Huang et al., 2019). Upon bacterial infection, expression of the biosynthetic genes of antioxidant compounds appeared to



**Fig. 5.** The top 15 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in which differentially expressed genes (DEGs) are participating. Each protein ID of DEGs was converted to KEGG gene ID using a biological DataBase network (bioDBnet, <https://biodbnet-abcc.ncifcrf.gov/>) and the converted KEGG gene IDs were used for the KEGG pathway analysis (KEGG Mapper-Color). The top 15 pathways were presented here with the exact number of genes. The whole KEGG pathways related to each DEG were listed in Supplementary Table 4.



be increased as expected. For instance, the expression of genes encoding enzymes involved in ascorbate biosynthesis, such as mannose-6-phosphate isomerase 1 (EC: 5.3.1.8, Entrez\_Gene\_ID: 100815644), phosphomannomutase (EC: 5.4.2.8, Entrez\_Gene\_ID: 732605), and mannose-1-phosphate guanylyltransferase 1-like (EC: 2.7.7.13, Entrez\_Gene\_ID: 100777120), increased during infection (Supplementary Table 4). In addition, numerous glutathione S-transferases, which function as one of the major detoxifying enzymes by conjugating tripeptide ( $\gamma$ -Glu-Cys-Gly) glutathione to various hydrophobic and electrophilic substrates (Liu et al., 2015a), were actively transcribed in the leaves infected with *PssB728a* (Supplementary Table 4).

Furthermore, a variety of genes related to an ER stress response, which occurs when the capacity of the ER to fold proteins becomes saturated and leads to activation of stress-adaptive response called unfolded protein response (UPR) (Bao and Howell, 2017; Ruberti et al., 2015), showed the altered transcription patterns under *PssB728a* infection. Three soybean orthologs of an ER stress sensor, the ER-resident transmembrane protein inositol-requiring enzyme 1 (serine/threonine-protein kinase/endoribonuclease IRE1, EC: 2.7.11.1 or EC: 3.1.26, Entrez\_Gene\_IDs: 100785904, 100788389, 102661527), reduced their mRNA expression upon bacterial infection (Supplemental Table 4). An ortholog of another ER-resident transmembrane protein ER stress sensor, PERK (eukaryotic translation initiation factor 2- $\alpha$  kinase 4/ eIF-2- $\alpha$  kinase GCN2 isoform X1 (EC:2.7.11.1, Entrez\_Gene\_ID: 100797996), which has been characterized as an ER stress sensor in animal cells but not identified experimentally in plant cells (Park and Park, 2019), also showed the reduced expression in infected leaves. In contrast, several ER-stress responsive genes and genes whose products participate in the protein secretion process exhibited higher expression upon bacterial infection (Supplemental Table 4). An ER stress-inducing compound, tunicamycin enhanced the susceptibility to *P. syringae* pv. tomato DC3000 in *Arabidopsis* while gene expression of *PR1* and FLS2-mediated PTI such as callose deposition increased (Chakraborty et al., 2017). *ire1* and *bzip60* mutants, which are deficient in IRE1, an ER stress sensor activating UPR and its splicing target bZIP-transcription factor, bZIP60, respectively, were more susceptible to *P. syringae* pv. *maculicola* (Moreno et al., 2012). Consistently, knock-down tobacco plants of *IRE1* and *bZIP60* via virus-induced gene silencing were more sensitive to the necrotic fungal pathogen, *Alternaria alternata* (Xu et al., 2019). The transcriptome profile of soybean plants infected with *PssB728a* suggests that ER-mediated

biological process is significantly affected in a compatible interaction (Saijo et al., 2009).

Among the top 15 pathways (Fig. 5), we analyzed further details about plant hormone signal transduction [5], MAPK signaling pathway-plant [10], and plant-pathogen interaction [11]. Genes participating in auxin- and cytokinin-mediated signaling were up-regulated in soybean leaves under *PssB728a* infection, whereas ethylene (ET)-, jasmonic acid (JA)-, and salicylic acid (SA)-mediated signaling seemed to be down-regulated in *PssB728a*-infected leaves (Supplementary Fig. 1). In the hormone-biosynthetic aspects, transcript levels of genes participating in the last half of  $\alpha$ -linolenic acid metabolic pathway for JA biosynthesis decreased by more than 2-folds in the infected plant (Supplementary Fig. 2). On the other side, soybean orthologs encoding three critical enzymes for the ET biosynthesis, S-ADENOSYLMETHIONINE SYNTHETASE (SAM), 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE (ACS), and AMINOCYCLOPROPANECARBOXYLATE OXIDASE (ACO) were actively transcribed under *PssB728a* infection (Supplementary Table 4). Additionally, a putative ortholog of *Arabidopsis* *ISOCHORISMATE SYNTHASE1 (ICS1)*, *ICS* was strongly transcribed in the infected leaves (EC:5.4.4.2, Entrez\_Gene\_ID: 100799025) albeit genes involved in shikimate pathway did not show any trends on transcriptional change under infection (Supplementary Table 4). Note that we do not have any experimental clues on the dynamics of these hormones in the infected plants. To measure hormone levels during infection will be necessary to understand the compatible interaction. Taken together, we assume that *PssB728a* successfully suppresses plant immune signaling activated by ET, JA, and SA and employs growth-related hormone signaling for their colonization.

MAPK signal cascades consisting of three sequential serine/threonine kinases (MAPKKK  $\rightarrow$  MAPKK  $\rightarrow$  MAPK) transmit environmental and developmental signals upon a range of stresses, including pathogen infection, heat, freezing, ROS, drought, and wounding (Meng and Zhang, 2013). Various hormones such as auxin, abscisic acid (ABA), JA, SA, and ET affect the MAPK signaling pathway (Jagodzik et al., 2018). Upon *PssB728a* infection, ABA receptors PYRABACTIN RESISTANCE 1 (PYR1)/PYRABACTIN RESISTANCE 1-LIKE (PYL) and its downstream signaling components type 2C protein phosphatases (PP2C), negative regulators of ABA signaling, displayed higher mRNA expression. In contrast, the expressions of MAPK signaling components were reduced, probably leading to a non-efficient and unsuccessful stress-adaptive response with disease increase (Supplementary

Fig. 3). MAPK pathway relays signals from membrane-residing immune receptors to downstream components for defense activation in plants (Thulasi Devendrakumar et al., 2018). Most of the MAPKKs, MAPKKs, and MAPKs detected in this study were down-regulated in the transcript levels on day 5 after *PssB728a* infection (Supplementary Fig. 3). Besides MAPK signaling cascades, genes encoding extracellular (co)receptors, CYCLIC NUCLEOTIDE GATED CHANNELS, and Ca<sup>2+</sup>-signaling proteins were weakly expressed in the infected leaves of soybean (Supplementary Fig. 4). However, a soybean ortholog of FLA-GELLIN-SENSITIVE 2 (FLS2) showed increased mRNA expression in the soybean leaves infected with *PssB728a*. However, its downstream pathways, MEKK1-MKK4/5-MPK3/6 and MPK4, reduced their transcription. In addition, soybean homologous genes of well-identified defense regulators in *Arabidopsis* and tomato, such as *EDS1*, *RIN4*, *SGT1*, and *Pti1/5/6*, displayed reduced mRNA levels in the infected leaves (Supplementary Fig. 4). This KEGG pathway analysis suggests that the plant immune responses of soybean cv. Kwangan are compromised by *PssB728a*.

In this study, we developed a pathosystem between soybean cv. Kwangan and *PssB728a*, a causal pathogen of bacterial brown spot disease, and presented the transcriptional changes in soybean leaves on day 5 after *PssB728a* infection. Unexpectedly, mRNA expression of homologous genes to well-identified immune-related genes in model and other crop plants decreased or was not dramatically altered in soybean leaves on day 5 after infection. In contrast, primary and secondary metabolisms underwent a change in *PssB728a*-infected conditions. Thus, these analyses represent the transcriptional changes at the late phase of a compatible interaction and reflect the suppression of plant immunity in susceptible plants by virulent pathogen infection. Furthermore, this study identifies large number of candidates for future functional analyses and provides a foundation to understand molecular mechanisms implicated in the compatible interaction of a susceptible Korean soybean cultivar and bacterial brown spot disease.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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### Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (<http://www.ppjonline.org/>).

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